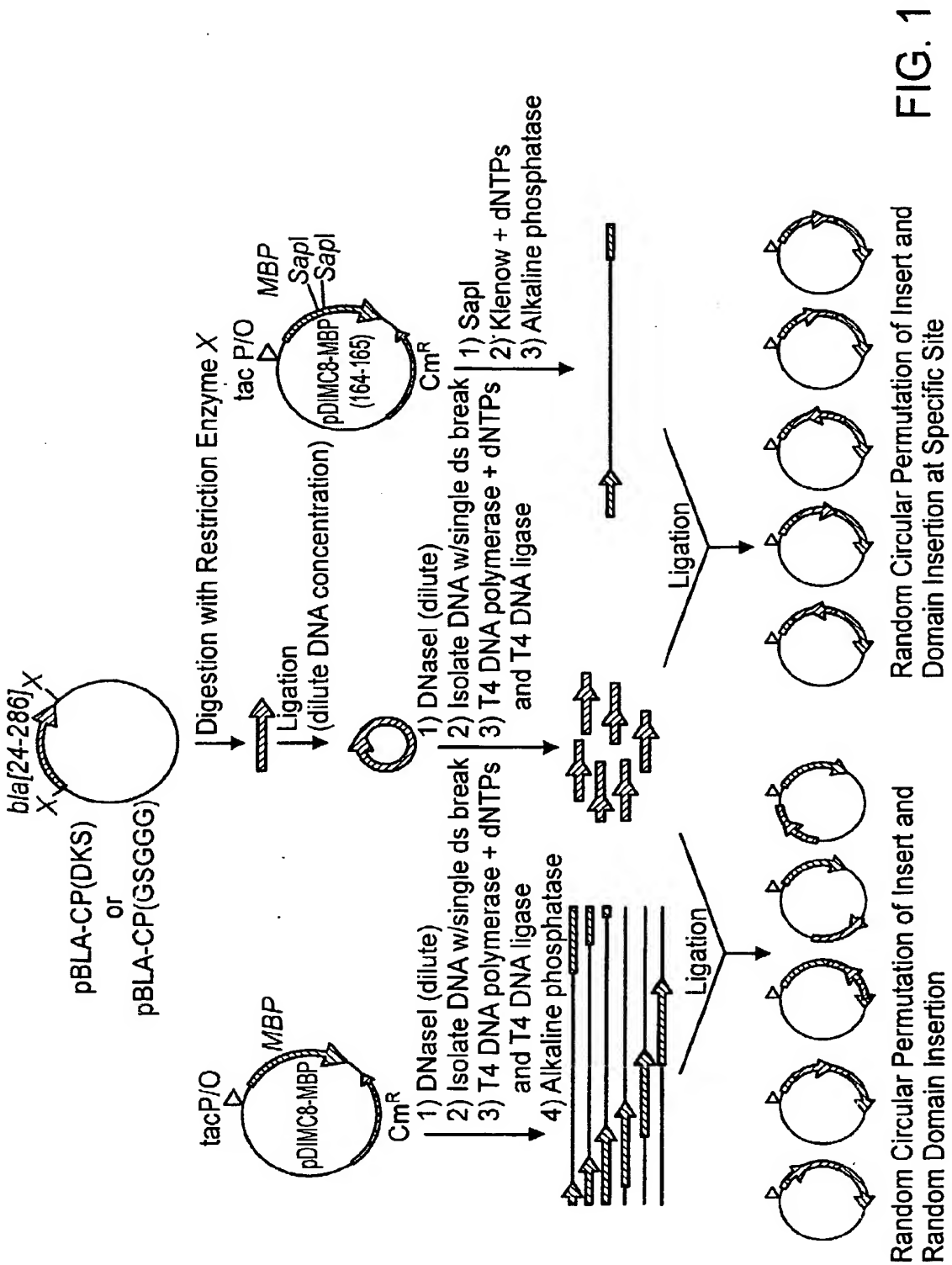

bla[24-286]
X
X
pBLA-CP(DKS)
or
pBLA-CP(GSGGG)
Digestion with Restriction Enzyme X
Ligation
(dilute DNA concentration)
tacP/O
MBP
pDIMC8-MBP
CmR
tac P/O
MBP
SapI
SapI
pDIMC8-MBP
(164-165)
CmR
1) DNaseI (dilute)
2) Isolate DNA w/single ds break
3) T4 DNA polymerase + dNTPs
and T4 DNA ligase
4) Alkaline phosphatase
1) DNaseI (dilute)
2) Isolate DNA w/single ds break
3) T4 DNA polymerase + dNTPs
and T4 DNA ligase
1) SapI
2) Klenow + dNTPs
3) Alkaline phosphatase
Ligation
Ligation
Random Circular Permutation of Insert and
Random Domain Insertion
Random Circular Permutation of Insert and
Domain Insertion at Specific Site

FIG. 1

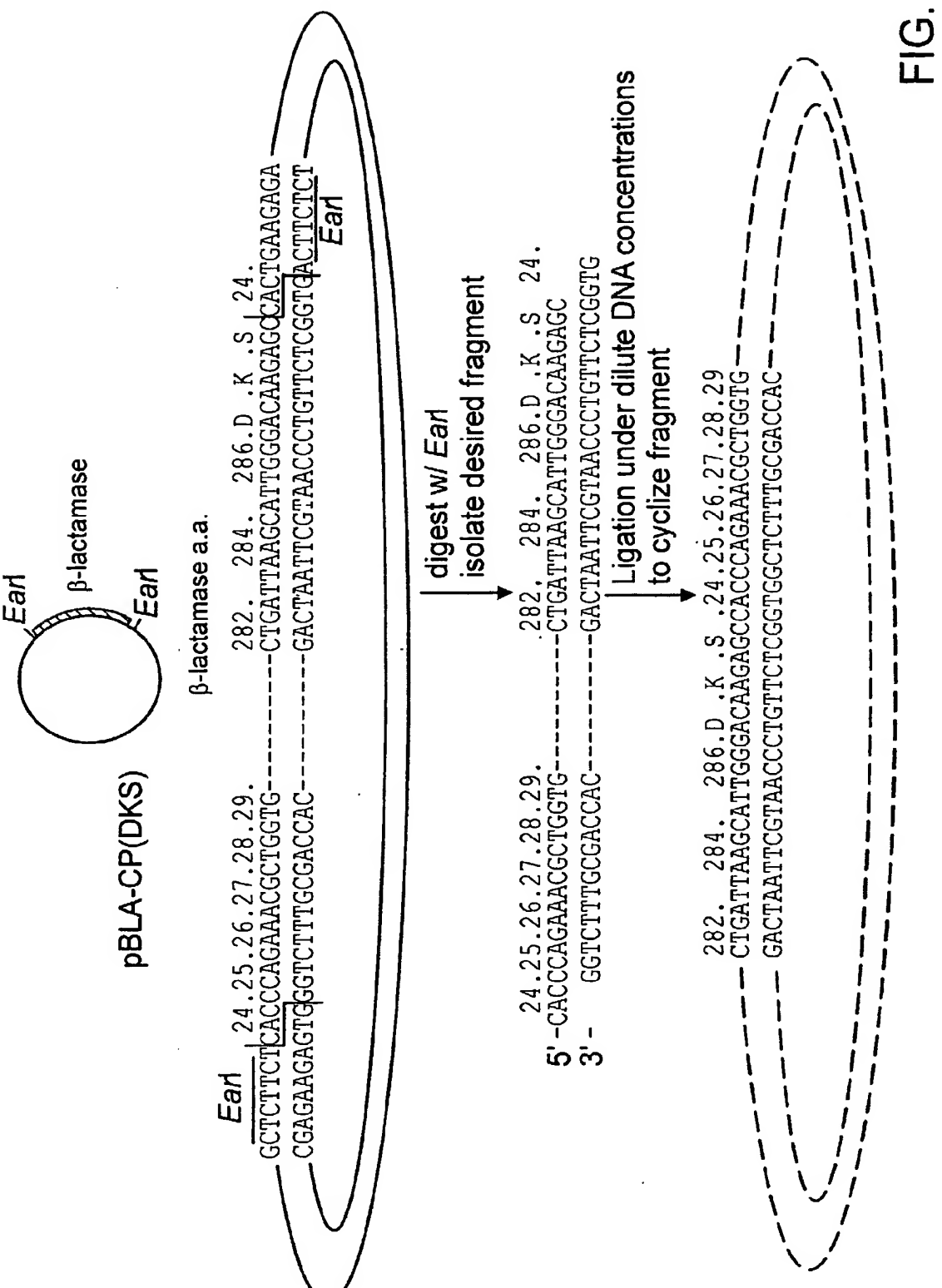

pBLA-CP(DKS)
β-lactamase
EarI
EarI
β-lactamase a.a.
282. 284. 286.D .K .S 24.
24.25.26.27.28.29.
EarI
GCTCTTCTCACCCAGAAACGCTGGTG----------CTGATTAAGCATTGGGACAAGAGCCACTGAAGAGA
CGAGAAGAGTGGGTCTTTGCGACCAC----------GACTAATTCGTAACCCTGTTCTCGGTGACTTCTCT
EarI
digest w/ EarI
isolate desired fragment
282. 284. 286.D .K .S 24.
24.25.26.27.28.29.
5'-CACCCAGAAACGCTGGTG----------CTGATTAAGCATTGGGACAAGAGC
3'- GGTCTTTGCGACCAC----------GACTAATTCGTAACCCTGTTCTCGGTG
Ligation under dilute DNA concentrations
to cyclize fragment
282. 284. 286.D .K .S 24.25.26.27.28.29
CTGATTAAGCATTGGGACAAGAGCCACCCAGAAACGCTGGTG
GACTAATTCGTAACCCTGTTCTCGGTGGCTCTTTGCGACCAC

FIG. 2

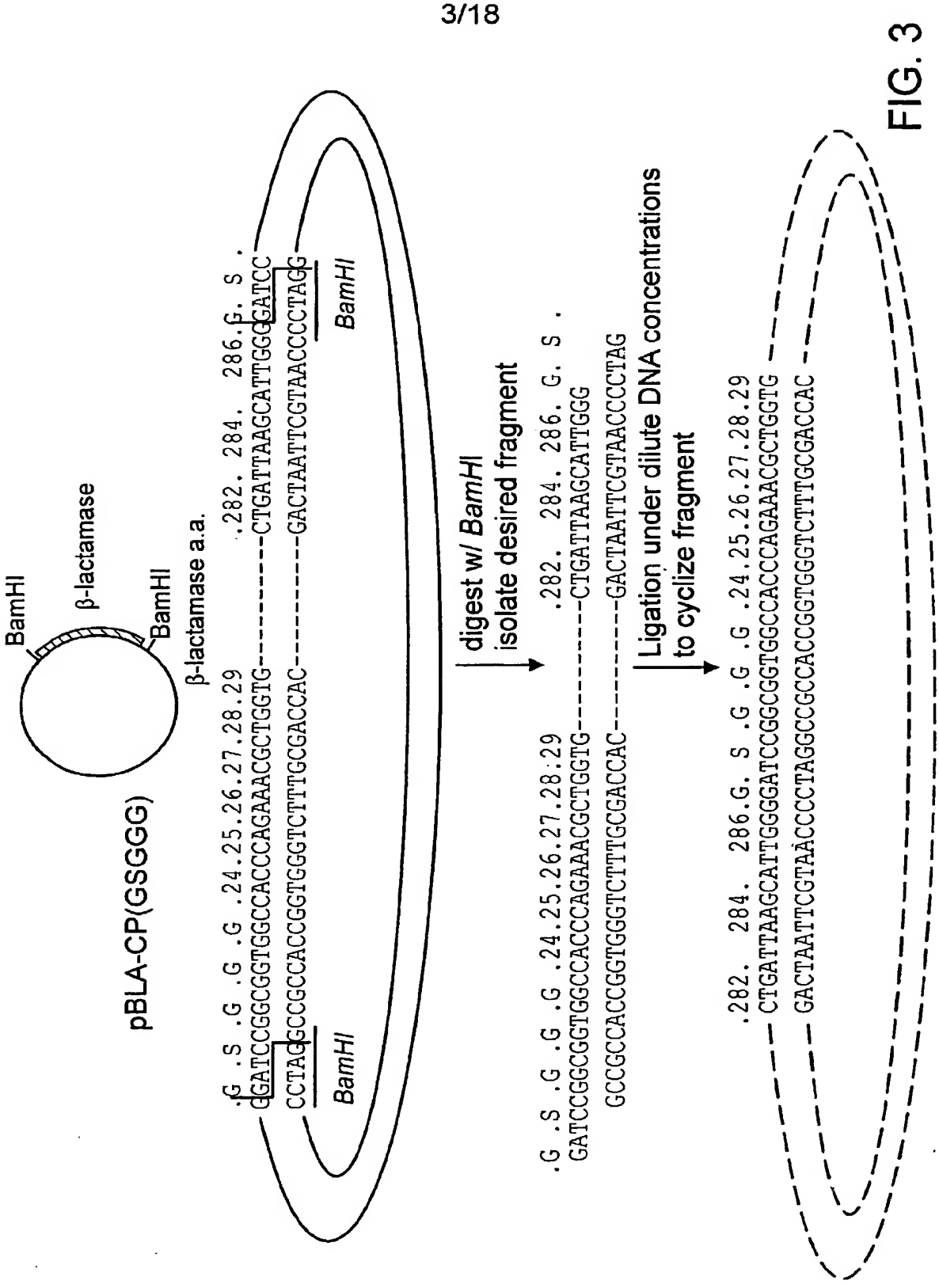

BamHI
β-lactamase
BamHI
pBLA-CP(GSGGG)
β-lactamase a.a.
.G .S .G .G .G .24.25.26.27.28.29 .282. 284. 286.G. S .
GGATCCGGCGGTGGCCACCCAGAAACGCTGGTG----------CTGATTAAGCATTGGGGATCC
CCTAGGCCGCCACCGGTGGGTCTTTGCGACCAC----------GACTAATTCGTAACCCCTAGG
BamHI
BamHI
digest w/ BamHI
isolate desired fragment
.G .S .G .G .G .24.25.26.27.28.29 .282. 284. 286. G. S .
GATCCGGCGGTGGCCACCCAGAAACGCTGGTG----------CTGATTAAGCATTGGG
GCCGCCACCGGTGGGTCTTTGCGACCAC----------GACTAATTCGTAACCCCTAG
Ligation under dilute DNA concentrations
to cyclize fragment
.282. 284. 286.G. S .G .G .G .24.25.26.27.28.29
CTGATTAAGCATTGGGGATCCGGCGGTGGCCACCCAGAAACGCTGGTG
GACTAATTCGTAACCCCTAGGCCGCCACCGGTGGGTCTTTGCGACCAC

FIG. 3

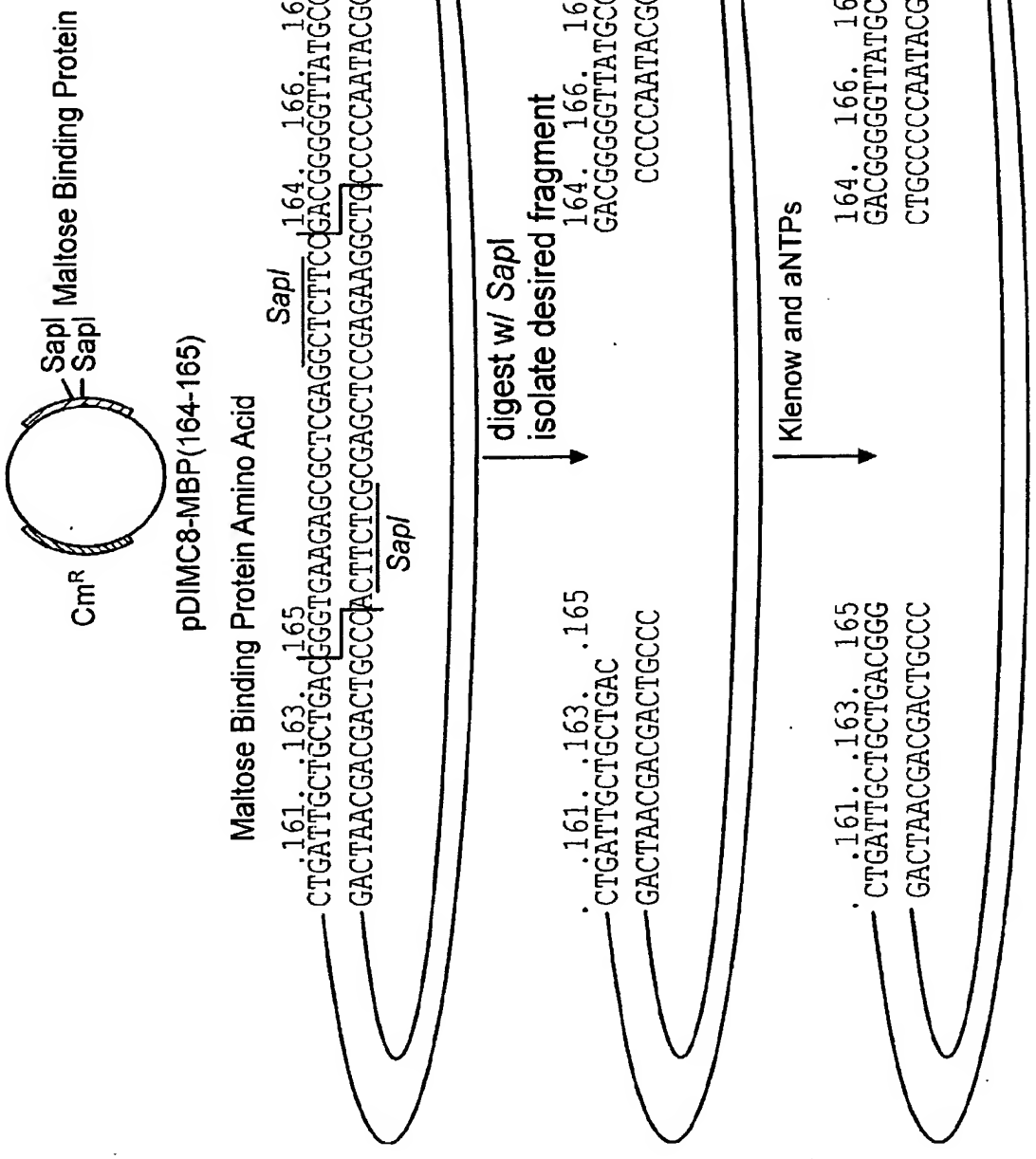

SapI
SapI
Maltose Binding Protein
Cm^R
pDIMC8-MBP(164-165)
Maltose Binding Protein Amino Acid
161 163 165
CTGATTGCTGCTGACGGGTGAAGAGGCGCTCGAGGCTCTTCGACGGGGGTTATGCGTTC
GACTAACGACGACTGCCCACTTCTCGCGAGCTCCGAGAAGGCTGCCCCCAATACGCAAG
SapI
SapI
digest w/ SapI
isolate desired fragment
161 163 165
CTGATTGCTGCTGAC
GACTAACGACGACTGCCC
164 166 168
GACGGGGGTTATGCGTTC
CCCCAATACGCAAG
Klenow and aNTPs
161 163 165
CTGATTGCTGCTGACGGG
GACTAACGACGACTGCCC
164 166 168
GACGGGGGTTATGCGTTC
CTGCCCCCAATACGCAAG

FIG. 4

FIG. 5A Gene Transcription


FIG. 5B Signal Transduction

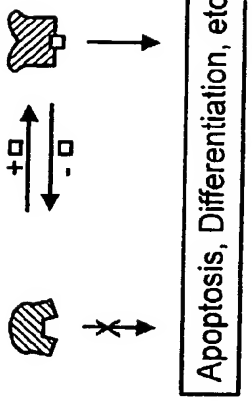
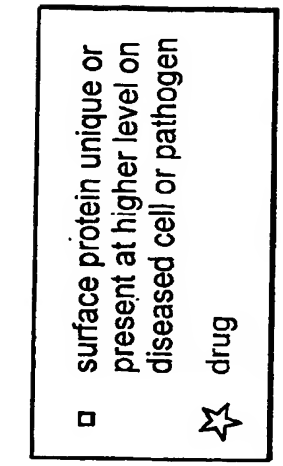
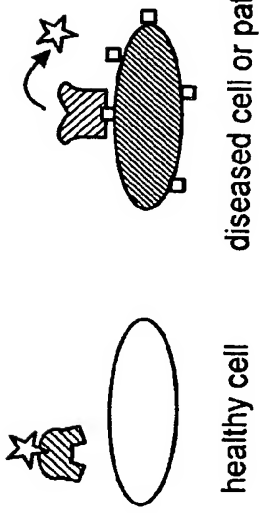



FIG. 5C Targeted Drug Delivery



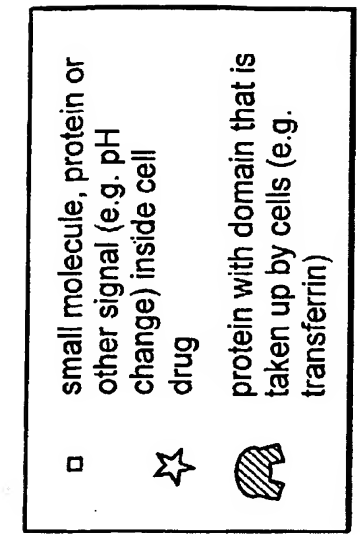



FIG. 5D Drug Transport

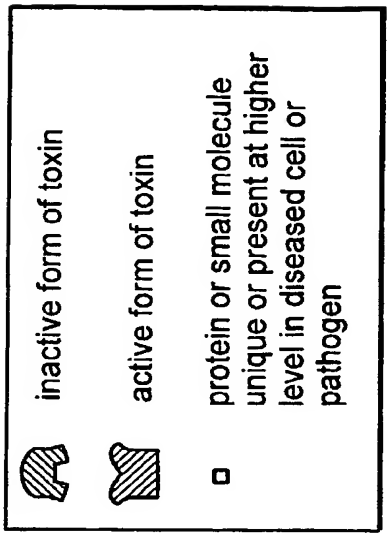



FIG. 5E Conditionally-active toxic proteins

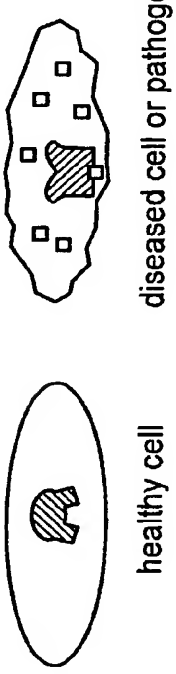



FIG. 5F Metabolic Engineering

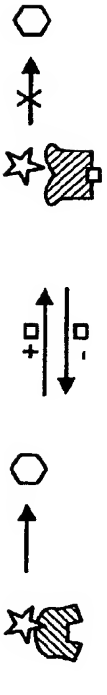
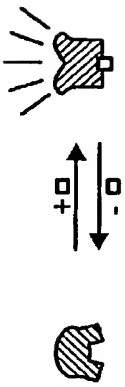


FIG. 5G Biosensors



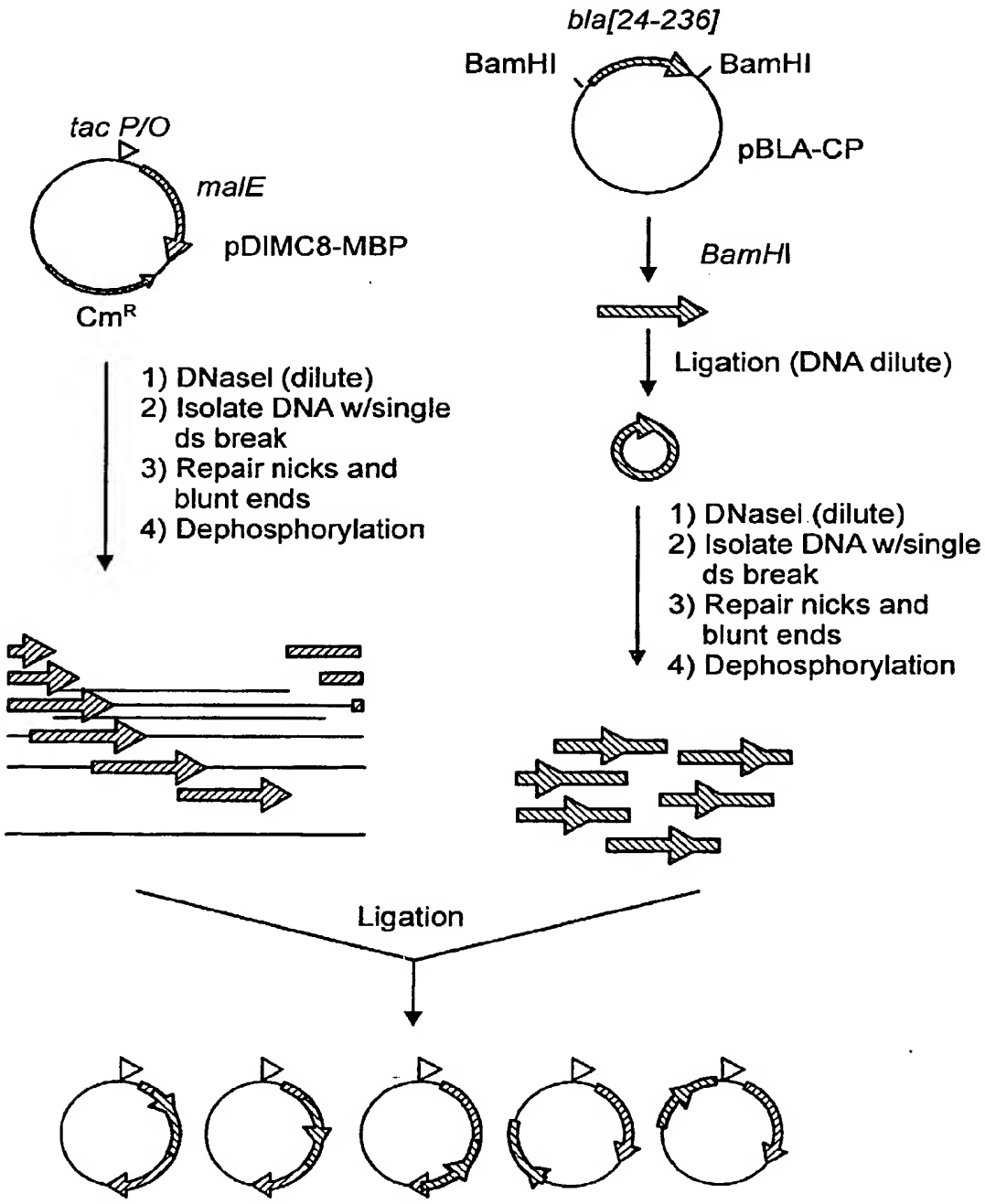

bla[24-236]
BamHI
BamHI
pBLA-CP
tac P/O
malE
pDIMC8-MBP
Cm^R
BamHI
Ligation (DNA dilute)
1) DNaseI (dilute)
2) Isolate DNA w/single ds break
3) Repair nicks and blunt ends
4) Dephosphorylation
1) DNaseI (dilute)
2) Isolate DNA w/single ds break
3) Repair nicks and blunt ends
4) Dephosphorylation
Ligation

FIG. 6A

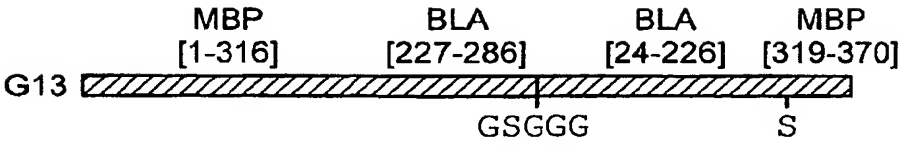

MBP
[1-316]
BLA
[227-286]
BLA
[24-226]
MBP
[319-370]
G13
GSGGG
S

FIG. 6B

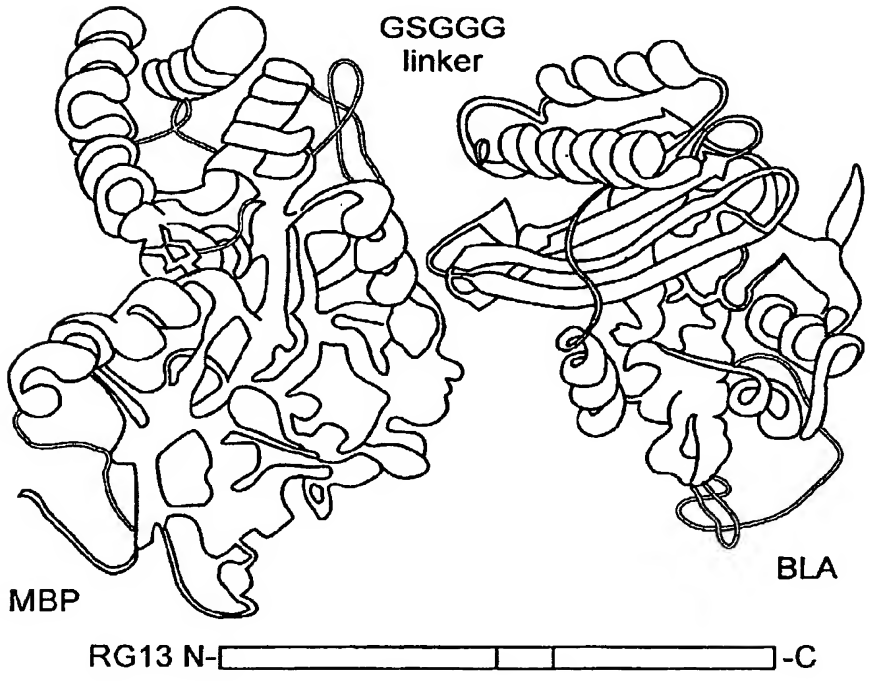

GSGGG
linker
BLA
MBP
RG13 N-
-C

FIG. 6C

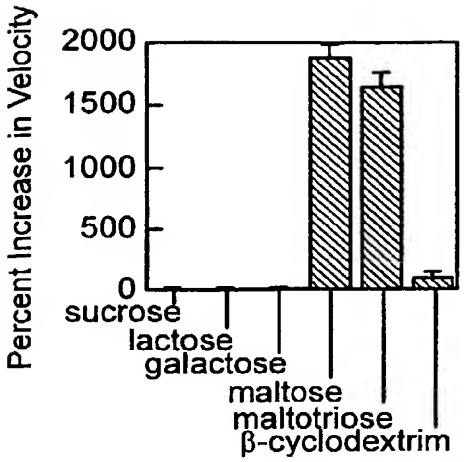



FIG. 7A

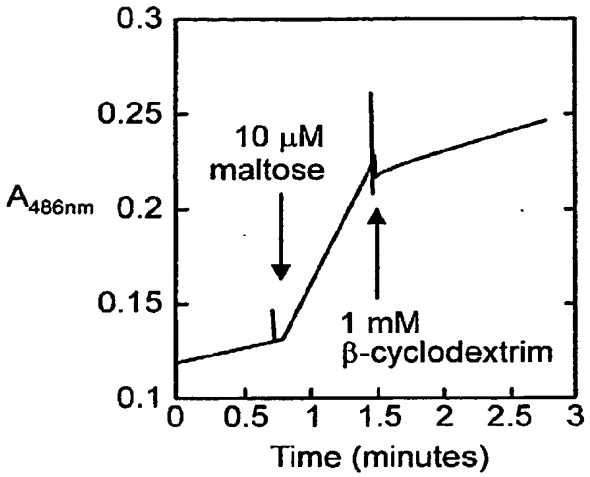



FIG. 7B

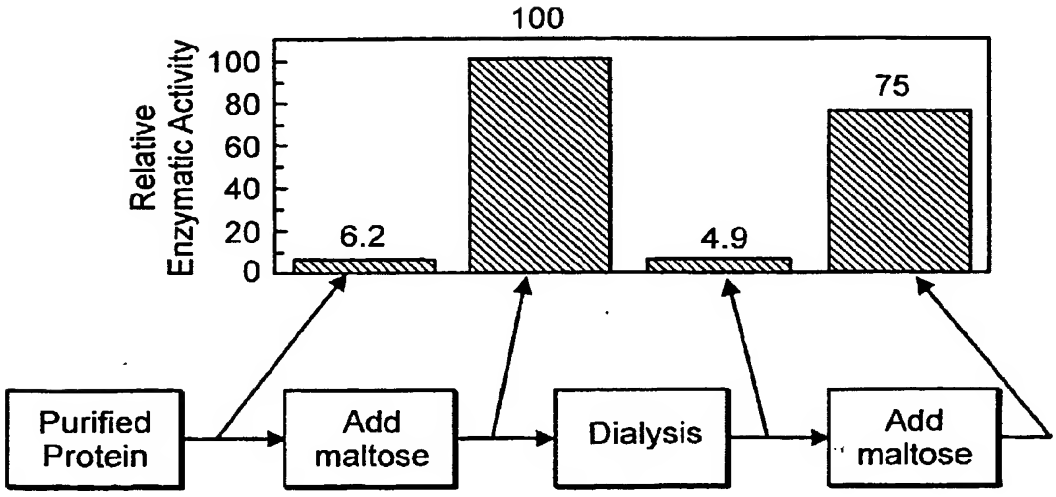



FIG. 7C

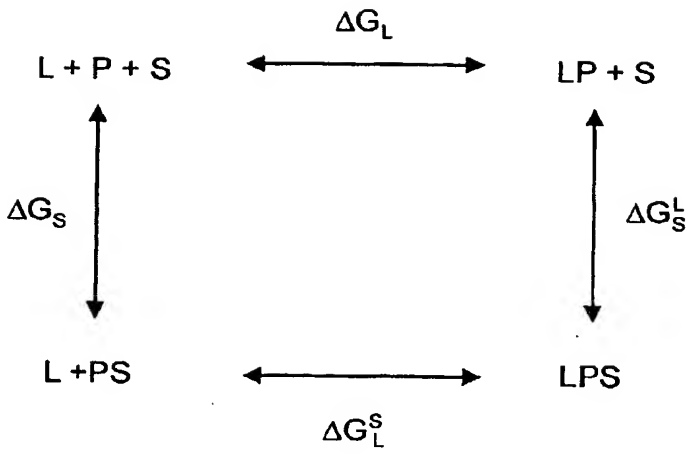



FIG. 8

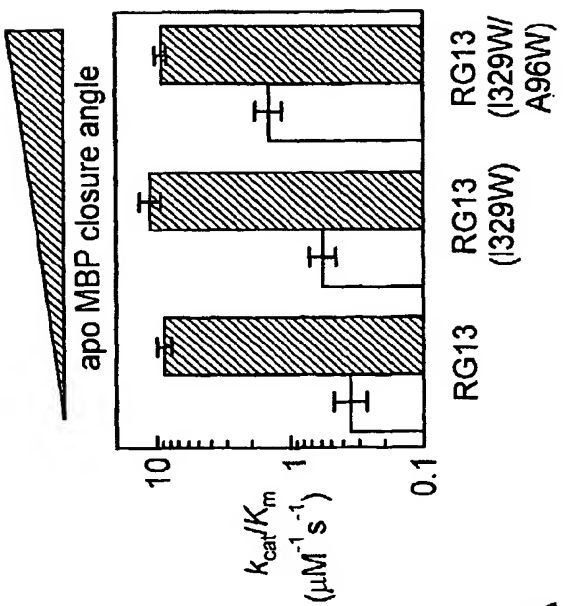



FIG. 9A

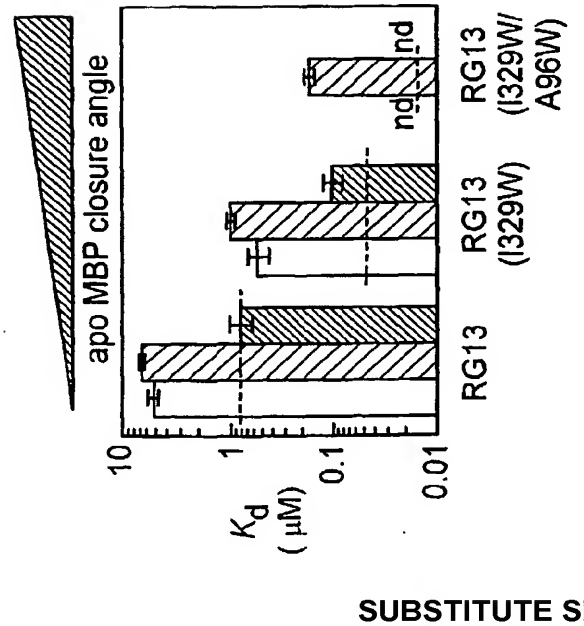



FIG. 9B

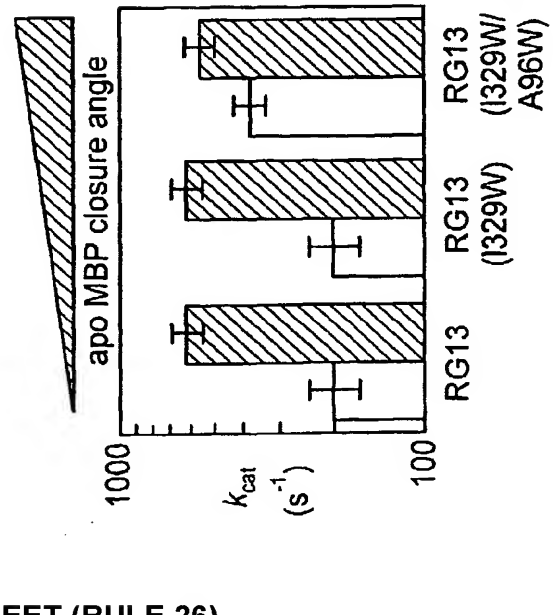



FIG. 9C

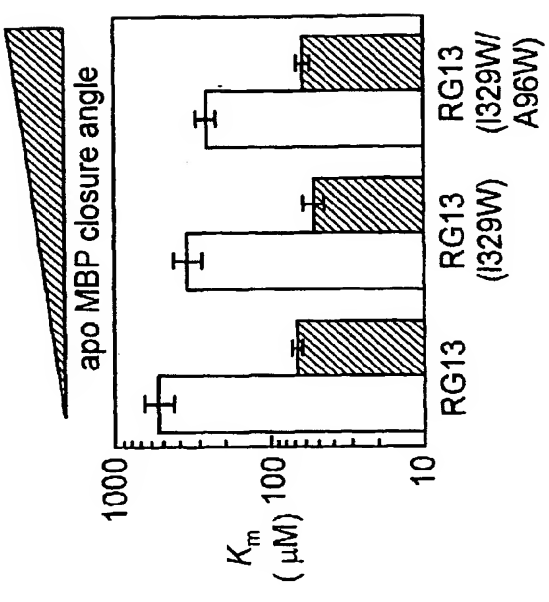



FIG. 9D

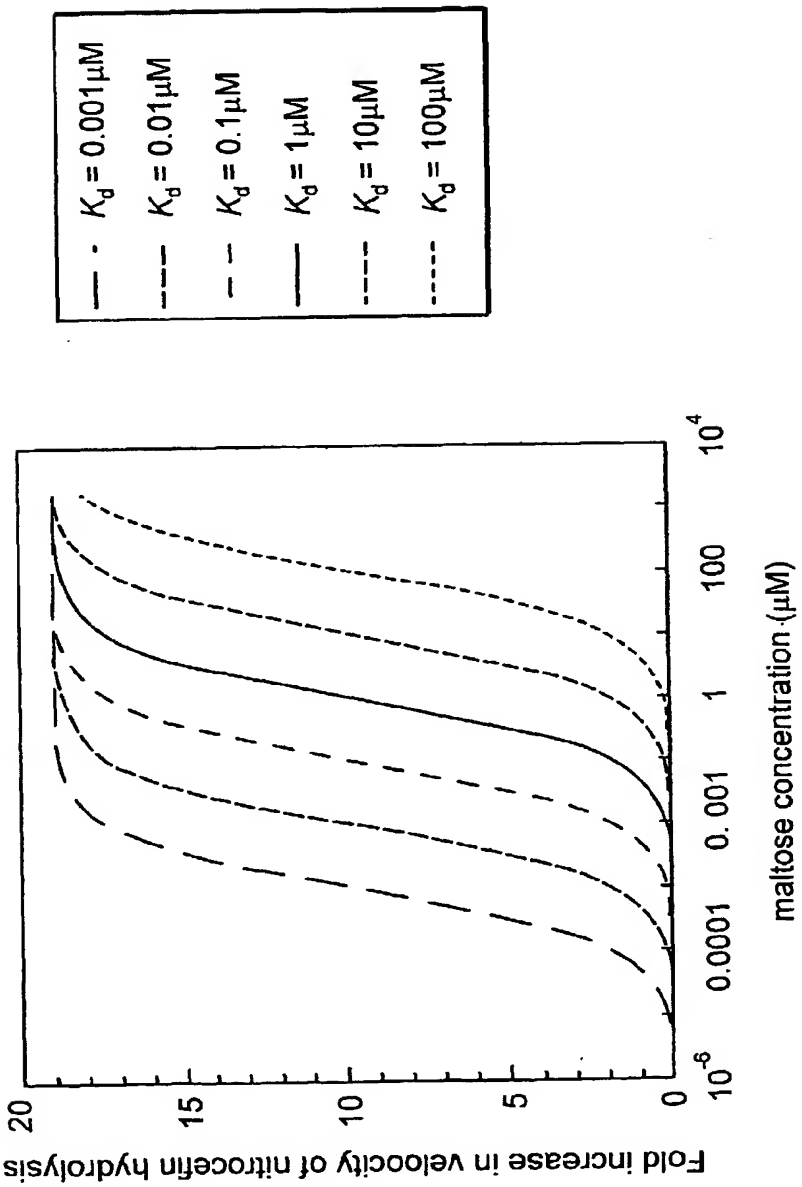

$K_d$ = 0.001μM
$K_d$ = 0.01μM
$K_d$ = 0.1μM
$K_d$ = 1μM
$K_d$ = 10μM
$K_d$ = 100μM
Fold increase in velocity of nitrocefin hydrolysis
0
5
10
15
20
$10^{-6}$
0.0001
0. 001
1
100
$10^{4}$
maltose concentration (μM)

FIG. 10

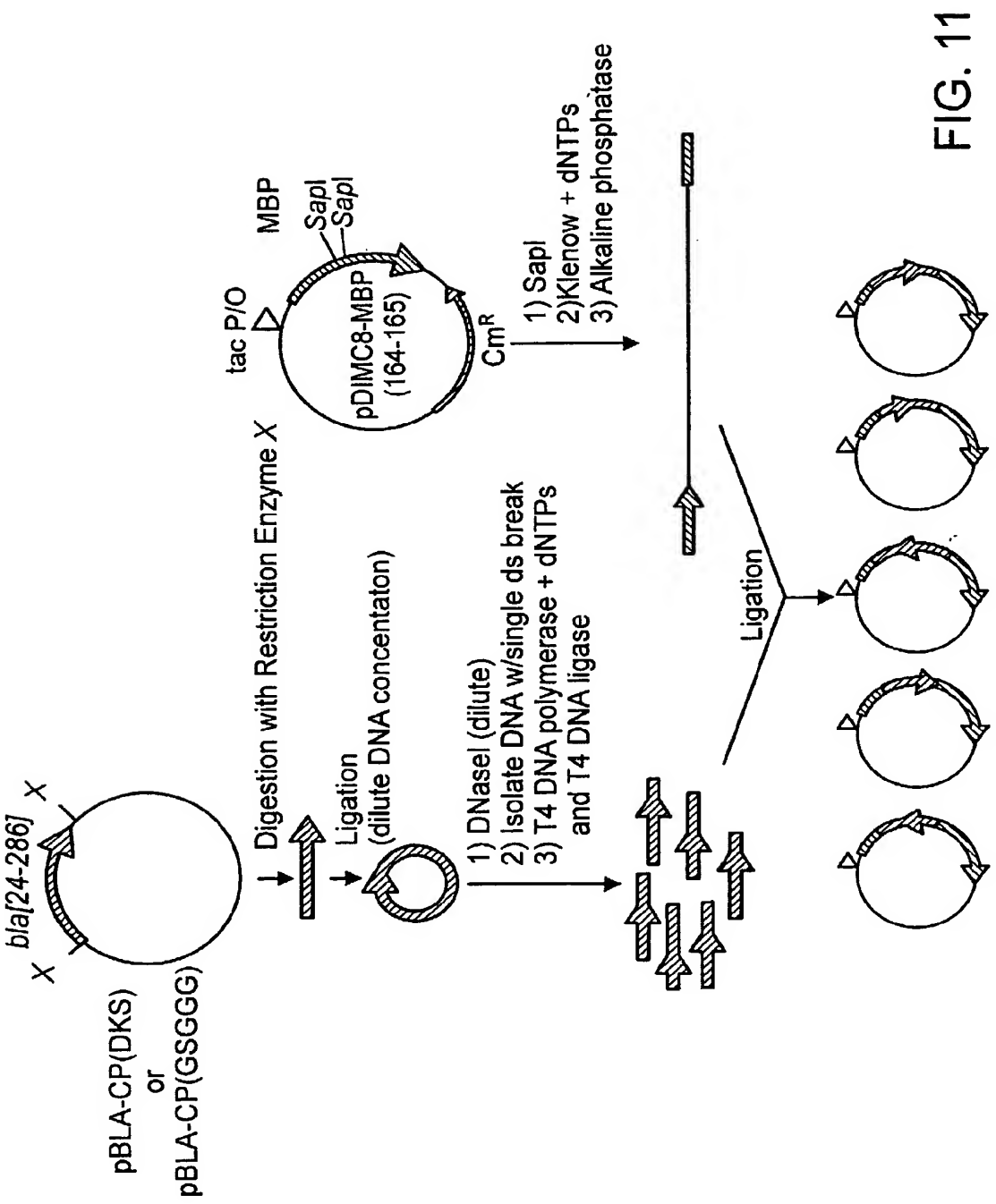

pBLA-CP(DKS)
or
pBLA-CP(GSGGG)
bla[24-286]
X
X
Digestion with Restriction Enzyme X
Ligation
(dilute DNA concentaton)
1) DNaseI (dilute)
2) Isolate DNA w/single ds break
3) T4 DNA polymerase + dNTPs
and T4 DNA ligase
tac P/O
MBP
SapI
SapI
pDIMC8-MBP
(164-165)
CmR
1) SapI
2)Klenow + dNTPs
3) Alkaline phosphatase
Ligation

FIG. 11

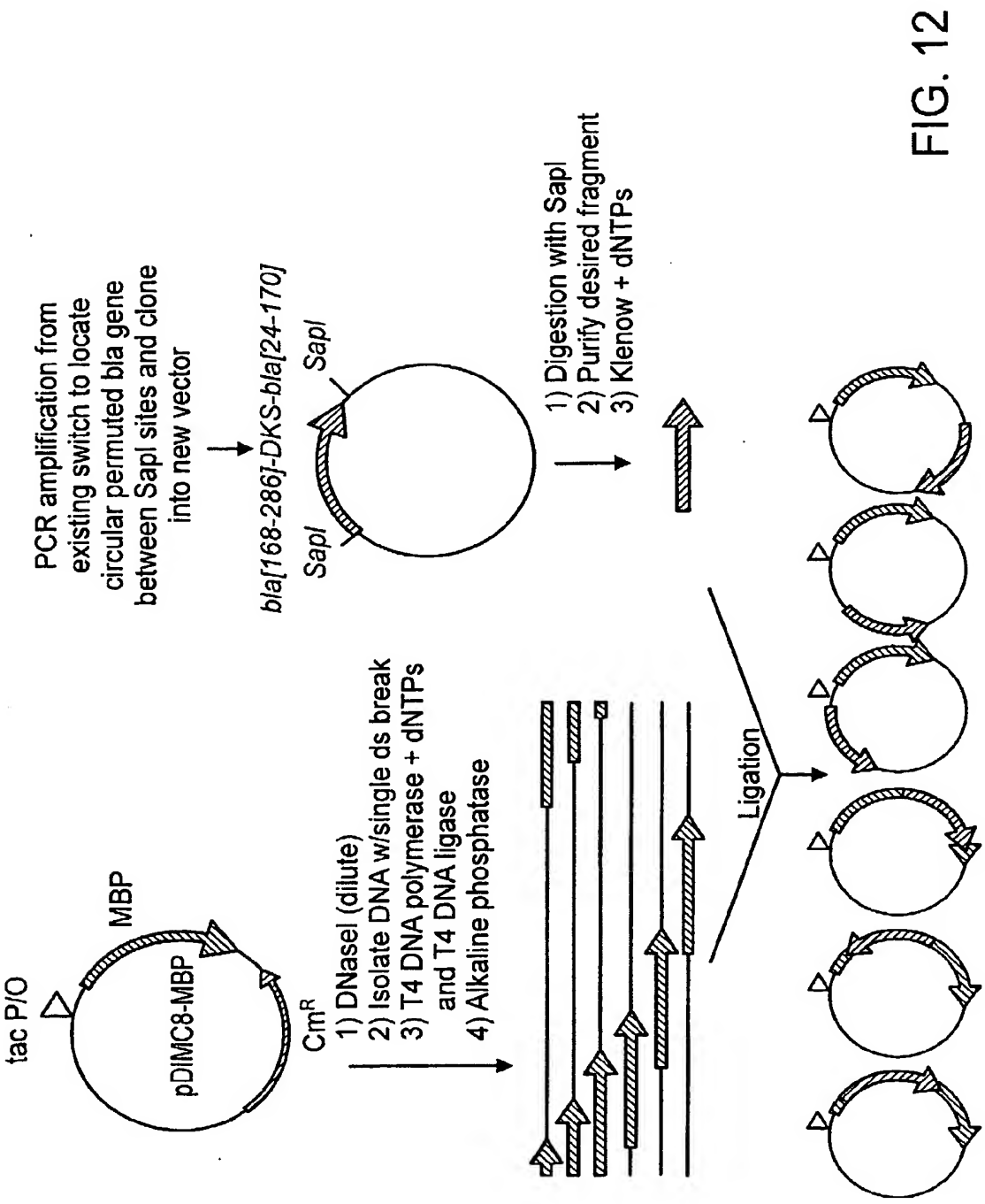

tac P/O
MBP
pDIMC8-MBP
Cm^R
1) DNaseI (dilute)
2) Isolate DNA w/single ds break
3) T4 DNA polymerase + dNTPs and T4 DNA ligase
4) Alkaline phosphatase
Ligation
1) Digestion with SapI
2) Purify desired fragment
3) Klenow + dNTPs
SapI
SapI
bla[168-286]-DKS-bla[24-170]
PCR amplification from existing switch to locate circular permuted bla gene between SapI sites and clone into new vector

FIG. 12

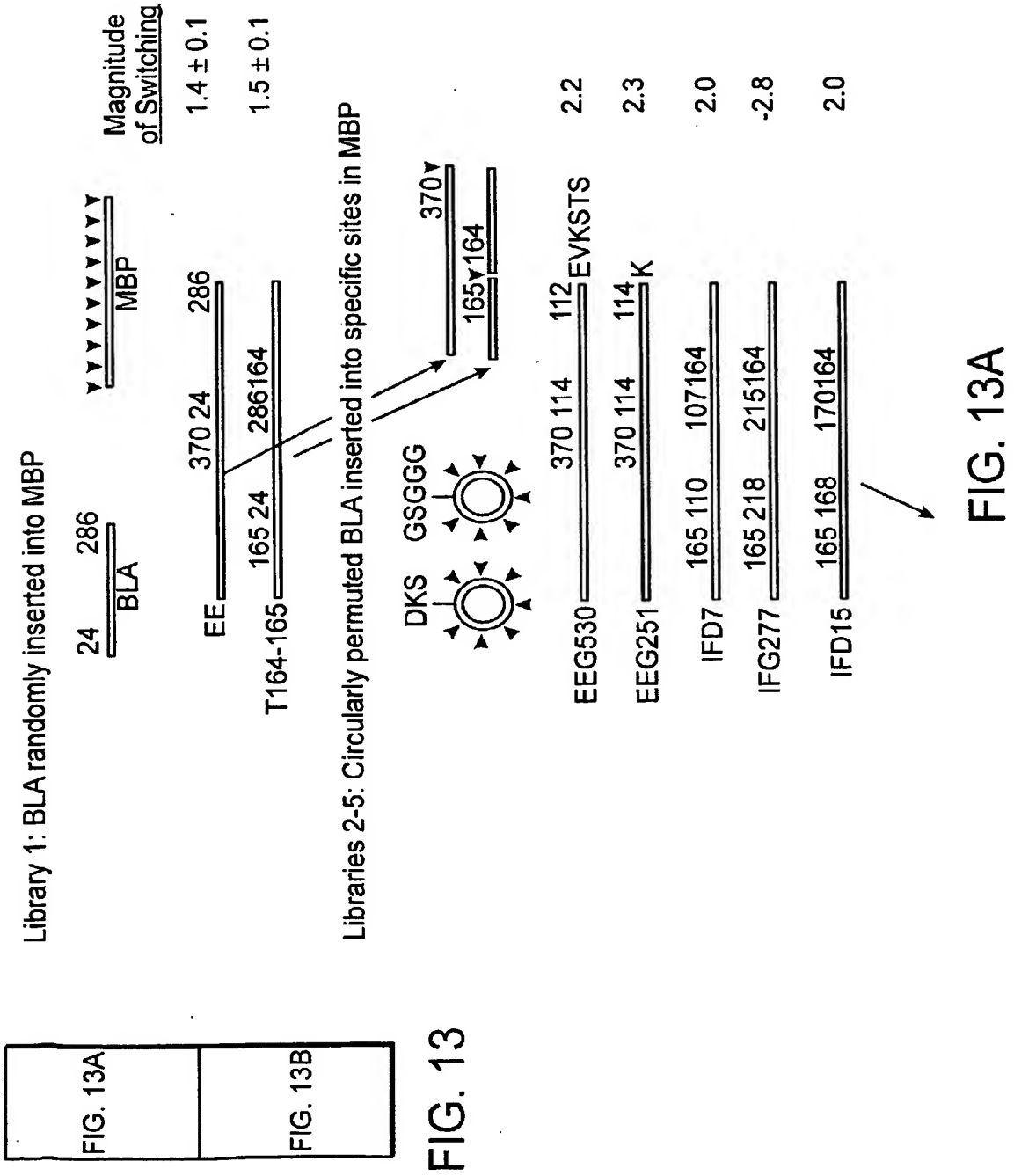



FIG. 13A

FIG. 13

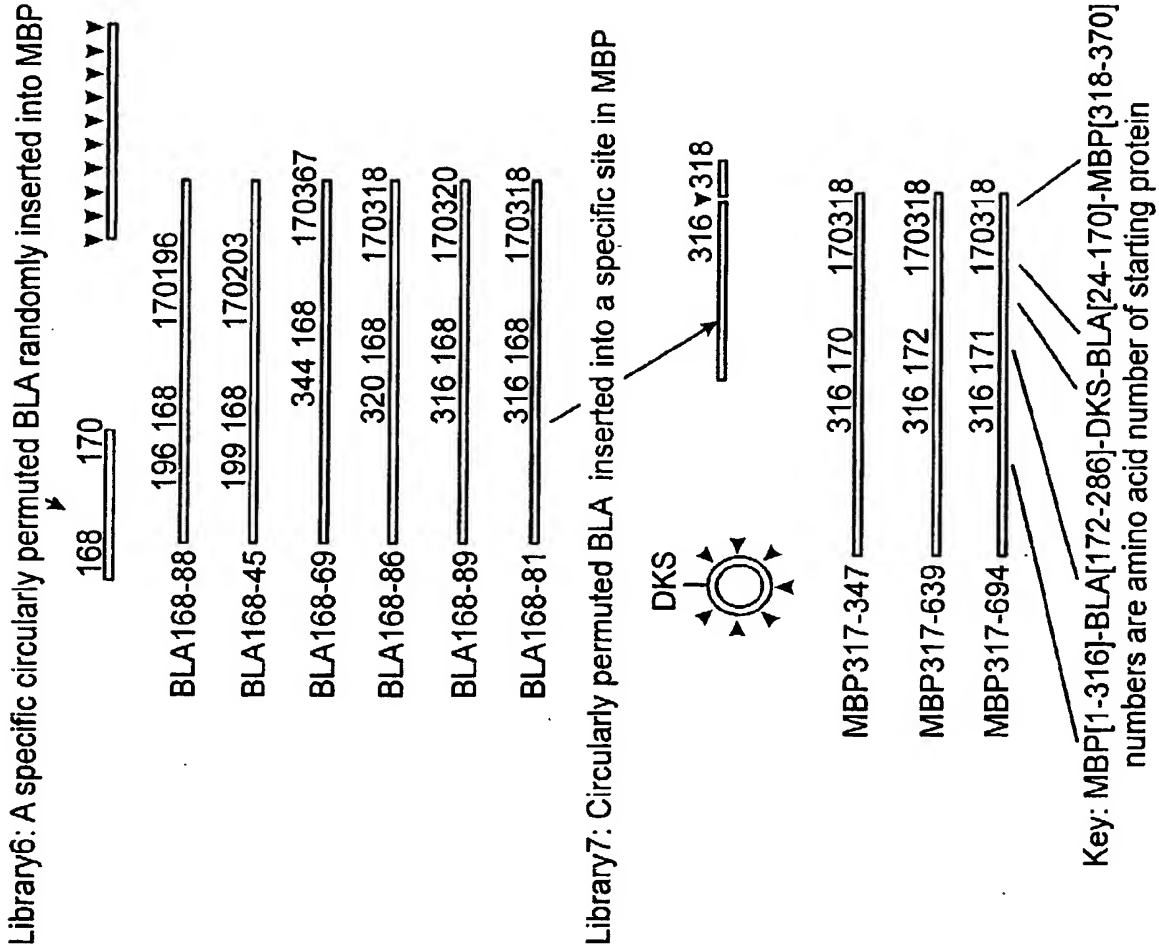

Library6: A specific circularly permuted BLA randomly inserted into MBP
168
170
BLA168-88 196 168 170196 1.6
BLA168-45 199 168 170203 -2.7
BLA168-69 344 168 170367 2.9 ± 0.5
BLA168-86 320 168 170318 16
BLA168-89 316 168 170320 11
BLA168-81 316 168 170318 92
Library7: Circularly permuted BLA inserted into a specific site in MBP
DKS
316 318
MBP317-347 316 170 170318 168 ± 41
MBP317-639 316 172 170318 221
MBP317-694 316 171 170318 94
Key: MBP[1-316]-BLA[172-286]-DKS-BLA[24-170]-MBP[318-370]
numbers are amino acid number of starting protein

FIG. 13B

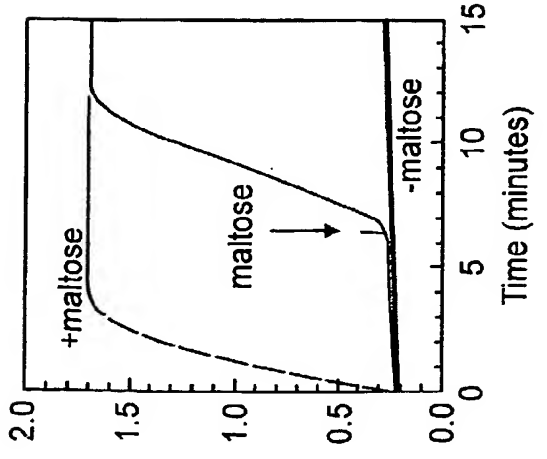



FIG. 14A

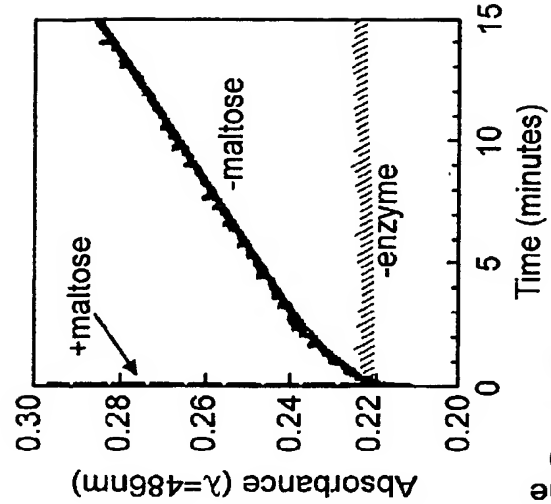



FIG. 14B




FIG. 14C

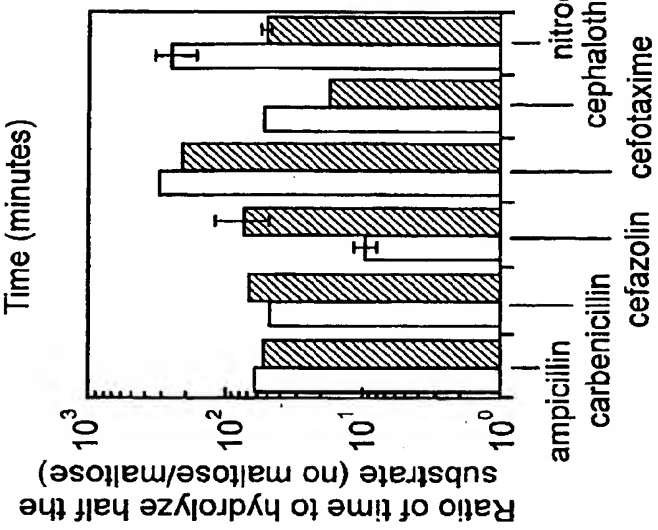



FIG. 14D

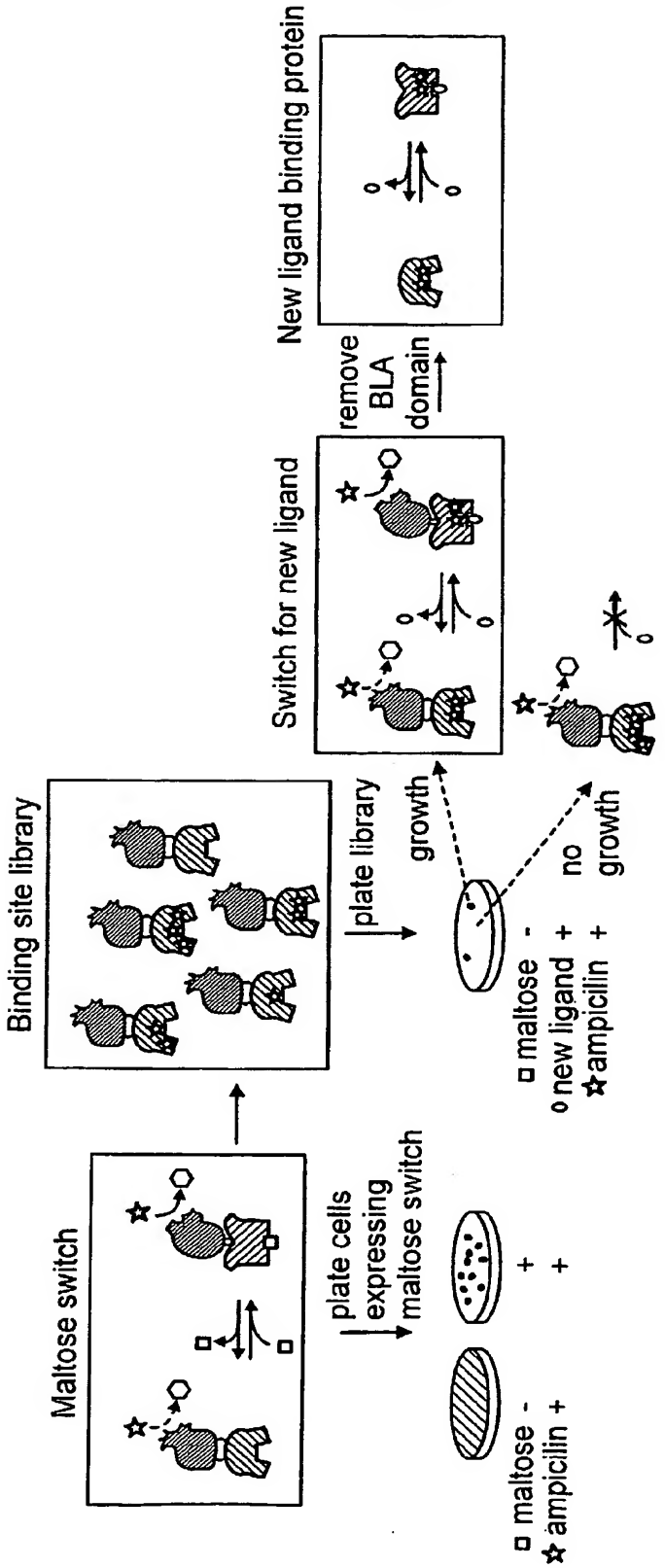

Maltose switch
plate cells expressing maltose switch
maltose -
ampicilin +
+
+
Binding site library
plate library
growth
maltose -
new ligand +
ampicilin +
no growth
Switch for new ligand
remove BLA domain
New ligand binding protein

FIG. 15